EXHIBIT A

-- PATENT APPLICATION --
-- Attorney Docket No. 25,835.11 --

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	M. L. Collins, et al.
Serial No.:	08/238,080
Filing Date:	May 3, 1994
Title:	TARGET AND BACKGROUND CAPTURE METHODS WITH AMPLIFICATION FOR AFFINITY ASSAYS
Art Unit:	1807
Examiner:	Dianne Rees, Ph.D.

DECLARATION OF DAVID H. PERSING, M.D., PH.D.

I, David H. Persing, M.D., Ph.D., declare and state as follows:

1. I am director of the Molecular Microbiology Lab of the Mayo Clinic, Rochester Minnesota. I have been employed by the Mayo Clinic since 1990. My work has been directed to the study of infectious diseases and includes the study of the application of nucleic acid hybridization assays in medical diagnostics.

2. I am a member of the Scientific Advisory Board of Vysis, Inc. I understand Vysis is a wholly owned company of Amoco Corporation, the owner of the subject patent application.

3. A copy of my curriculum vitae is attached as Exhibit 1. Briefly, I have been involved in molecular microbiology research since about 1978. Our laboratory is currently one of the premier centers for the diagnosis of infectious diseases by molecular methods. Our lab has pioneered techniques for pathogen discovery and contamination control, and has discovered several new pathogens as a result.

4. A list of my scientific publications is attached as Exhibit 2.

5. I have been provided with and have reviewed copies of the following documents:

(a) Patent Application U.S. Serial No. 08/238,080 entitled Target And Background Capture Methods With Amplification For Affinity Assays naming Collins et al. as inventors;

(b) A document entitled Preliminary Amendment And Response To Restriction Requirement dated December 5, 1995;

(c) U.S. Patent No. 4,851,331 entitled Method And Kit For Polynucleotide Assay Including Primer-Dependant DNA Polymerase naming Vary et al. (the "Vary patent") as inventors;

(d) European Patent Publication No. 0 139 489 entitled Sandwich Hybridization Method For Nucleic Acid Detection naming Hansen (the "Hansen" application) as the inventor;

(e) European Patent Publication No. 0 159 719 entitled Hybridization Method For the Detection Of Genetic Materials naming Rabbani et al. (the "Rabbani" application) as inventors;

(f) A transmittal letter from the Patent Office and accompanying Office Action Summary dated June 20, 1996;

(g) A transmittal letter from the Patent Office and accompanying Office Action Summary dated January 17, 1997; and

(h) The article "Sequence Capture-PCR Improves Detection of Mycobacterial DNA in Clinical Specimens," by Mangiapan et al., J. Clin. Microbiol., 34(5): 1209-1215 (1996).

6. I have reviewed claims 25 and 31 as presented in the Preliminary Amendment. I am informed that the inventions claimed in these claims were made on or before December 21, 1987.

7. I have been familiar with and been a practitioner of nucleic acid hybridization assays and various amplification techniques used with nucleic acid hybridization assays since about 1985. I have generally followed the literature of assay methods using nucleic acid hybridization since about 1985. As indicated in Exhibit 2, I have published a number of publications relating to these techniques and am a Editor-in-Chief of the reference text **Diagnostic Molecular Microbiology** PRINCIPALS AND APPLICATIONS.

8. I have been asked to consider whether the methods recited in claims 25 and 31 would have been obvious to those practicing in the field of nucleic acid hybridization assays and utilizing techniques for amplifying nucleic acids such as the polymerase chain reaction or PCR in light of the Vary patent, the Hansen application and the Rabbani application. In my opinion, the methods recited in claims 25 and 31 would not have been obvious to such practicioners in light of these references.

9. The Vary patent discloses a method for assaying for polynucleotides using primer dependent DNA polymerase. More particularly, the patent discloses

> a method for the determination of a target nucleotide sequence in the nucleic acid of a biological [sample] which comprises the steps:
> (a) contacting the sample with a probe polynucleotide of a sufficient length under conditions sufficient for the probe polynucleotide to bind to the target nucleotide sequence and form a hybrid having a double-stranded portion including the 3' end of the probe polynucleotide, with the sample nucleic acid strand extending in a 3' to 5' direction beyond the 3' end of the probe polynucleotide;
> (b) extending the probe polynucleotide strand of the hybrid beyond its 3' end in the 5' to 3' direction on the sample nucleic acid strand by incorporating nucleoside triphosphates from solution, a plurality of the nucleotides incorporated into the extended probe strand being detectably-modified nucleotides; and
> (c) detecting detectably-modified nucleotides which have been incorporated into probe polynucleotide strand as a measure of target nucleotide sequence in the biological sample. (Col. 1, line 54 - col. 2, line 6)

The primary feature of the invention is the selective incorporation of detectably labeled nucleotides into an elongation segment formed on a sample polynucleotide containing a target nucleotide sequence as a template and as an extension of a probe polynucleotide (which need not be labeled, but may contain a site for specific immobilization) as primer. (Col. 1, lines 47 - 53) More generally, the patent discloses a method for detecting a target polynucleotide in a sample comprising hybridizing a primer to the target polynucleotide, extending the primer, immobilizing the double-stranded polynucleotide product of the primer extension on a support, separating the double-stranded polynucleotide on the support from the sample and detecting the amplified polynucleotides. The double-stranded polynucleotide is then immobilized on a solid support and detected. Preferably, the double-stranded polynucleotide is separated from the sample for detection. (Col. 4, line 6 et seq.)

The patent does not disclose or suggest immobilizing and separating the target polynucleotide from the sample prior to hybridization of the primer to the target or primer extension.

Moreover, it is not even clear that the patent discloses amplification as that term is generally understood in the art and as is intended by claims 25 and 31. Target amplification generally means increasing the number of target polynucleotides manifold, typically exponentially. For example, amplification of nucleic acids by the polymerase chain reaction (PCR) follows primer extension with separation of the double-stranded primer extension product into single-stranded polynucleotides and repeating the process steps (hybridization of primer to target polynucleotide, primer extension and separation of the double-stranded product into more single-stranded polynucleotides) thereby increasing the population of detectable target polynucleotides exponentially. The Vary patent discloses only a single primer extension and detection of the extension product. Thus, in absolute terms, the number of polynucleotides actually detected by Vary's method can be no more than the number of target polynucleotides initially present in the sample. In contrast, the number of polynucleotides detected following target amplification can easily be more than a million times greater than the number of polynucleotides initially present in the sample.

10. The Hansen application discloses a method for detecting specific nucleic acids by providing the nucleic acid to be detected in single-stranded form and thereafter contacting it with a labeled nucleic acid probe specific for a given section of the nucleic acid strand. Additionally, a biotinylated nucleic acid probe specific for a different portion of the nucleic acid strand, is bonded to an avidin coated microparticle. The strand having the labeled probe hybridized to it is then mixed with the avidin coated microparticles. The probes are allowed to bind to the target nucleic acid so that the target becomes bound to the microparticle. The microparticles are then separated from the sample. The coupling of biotin to avidin is sufficiently strong that the targets remain bound to the microparticles and so are separated from the sample with the microparticles. The bound material is then assayed for the presence of the label signalling the presence of the target polynucleotide. (Page 2, lines 14 -33) The application discloses that the order of reaction among the assay components may be varied to suit the needs of the investigator. (Page 6, lines 17-21) The primary feature of the application appears to be the use of the strong binding characteristics of biotin and avidin in facilitating the separation of the target from the sample prior to detection. The application does not disclose or even consider target amplification as a part of its methods.

11. The Rabbani application discloses methods for the detection of target genetic material having a desired base sequence or gene, mutations and the deletion of a gene or base. The methods are based upon techniques which utilize two labeled single-stranded polynucleotide segments which are complementary to the same or opposite strands of the target material. These methods result in the formation of double or multi-hybrids. The multi-hybrids are detected by means of various labels. The application does not disclose or even consider target amplification as a part of its methods.

12. I have reviewed the arguments made by the Examiner in concluding that claims 25 and 31 as presented in the Preliminary Amendment are obvious in view of the Vary patent and the Hansen and Rabbani applications. I disagree with the Examiner's conclusion for the following reasons. As noted above, none of these references discloses any real teachings regarding the use of amplification in a nucleic acid hybridization assay. Accordingly, I believe it is inappropriate for the Examiner to apply the disclosure of these references to the use of amplification techniques for enhancing assay sensitivity. Although it may appear obvious today to apply these references as the Examiner has done, I believe that to do so overlooks or greatly oversimplifies the problems actually encountered by practitioners attempting to obtain highly sensitive assays using target amplification. I do not believe the methods of claims 25 and 31 were obvious in light of these references in December 1987.

It is necessary to keep in mind that the inclusion of target amplification to nucleic acid hybridization assays adds an additional, significant level of complexity to assay methodology. Additional materials are required; additional process steps are required; additional time is required; and additional cost is required to add amplification to conventional (unamplified) assay methodology. Those working with nucleic acid hybridization assays had no real incentive to add to their methods the complexity attendant to amplification unless the object targets were expected to be present in levels below the detection level of their conventional methods.

As techniques such as PCR were developed for amplifying nucleic acids, those practicing hybridization assays sought to incorporate the new amplification techniques into their methods. Initially, users and proponents of PCR believed that PCR was highly specific and could be made to selectively amplify the desired target in an otherwise complex sample system. Practitioners believed that adequate specificity could be imparted to the amplification by careful selection of the primers used in the amplification so that additional steps for isolating target prior to amplification were not required. Since the addition of such isolation steps would be costly and time consuming, would further complicate the assay and was generally believed to be unnecessary; those who were adding amplification to their nucleic acid hybridization assays had a strong incentive to avoid the addition of target

isolation steps to their hybridization assays. It was not until much later that it became apparent that non-specific amplification was occurring despite the careful selection of primers, i.e., that even careful selection of primers would not permit the selective amplification of a particular nucleic acid. I believe this realization did not occur until after December 1987.

13. I believe there is another reason why practicioners of hybridization assays were reluctant to use hybridization techniques to purify their intended targets from the initial sample system prior to amplification. This is the lack of complete binding efficiency in the initial target capture step. It is and was generally well understood that the binding efficiency of (capture) probe to target is substantially less than 100%. Thus, in sample systems where the presence of target nucleic acid is already known to be low, the lack of high binding efficiency meant that significantly less than the already low number of targets present in the sample would be captured and survive separation from the sample for amplification, thereby decreasing the already low amount of target available for detection. This concern over the low binding efficiencies of the capture step has persisted as is evidenced at page 127 in the section addressing Target Capture techniques from Chapter 6 of the reference text **Diagnostic Molecular Microbiology** (copyright 1993), attached as Exhibit 3. ("However, to date there are no published studies that demonstrate efficient capture and detection of fewer than 100 target molecules, ...")

Accordingly, I do not agree with the Examiner's conclusion that those incorporating amplification techniques into nucleic acid hybridization assays in or before December 1987 would have concluded that the methods claimed in claims 25 and 31 of the Preliminary amendment were obvious in light of the Vary patent, the Hansen application and the Rabbani application. To the contrary, coupled with the conventional understanding at that time (that careful selection of primers would permit adequate selectivity of the target and specificity in the amplification product), the practitioners' concern regarding imperfect binding efficiencies and the expected loss of real target before amplification occurred reinforced their incentive to avoid further complicating their assays by the addition of target separation steps to their assays.

I do not believe that the concerns of practitioners regarding imperfect binding efficiencies would have been overcome by the disclosure of the Hansen application which addressed a much more simplified assay system. There is nothing in Hanson application, for example, to suggest that practitioners should elect to first separate less than all of the scarce target from the sample before completing the assay.

14. Finally, I would also mention that the methods of Claims 25 and 31 have provided an additional advantage which was unexpected in or before December 1987. This is the elimination of amplification inhibitors normally present in the sample system. For example, as indicated by the article by Mangiapan, many clinical samples contain PCR inhibitors such as hemoglobin and sodium dodecyl sulfate. By separating the target from the sample prior to amplification, Applicants' methods effectively remove these inhibitors from the system enabling amplification to proceed optimally. This has an obvious beneficial effect on the overall assay.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledged that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of the Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

2/9/97
Date:

David H. Persing, M.D., Ph.D.